ANIMAL REPRODUCTIVE EFFICIENCY

Panel Manager – Dr. C. Richard Barb, USDA/ARS, R.B. Russell Agriculture Research Center
Program Director – Dr. Debora L. Hamernik

The primary objective of this program area is to increase our knowledge of reproductive biology in agriculturally important animals with the goal of increasing reproductive efficiency. This program supports innovative research on: (1) factors controlling ovarian function including follicular development, ovulation, and corpus luteum formation and function, (2) factors controlling male reproduction, (3) gamete physiology, including oogenesis and spermatogenesis, gamete maturation, and mechanisms regulating gamete survival *in vivo* or *in vitro*, (4) mechanisms involved in placental function, and (6) parturition, postpartum interval to conception, and neonatal survival.

Because alterations in animal behavior and animal well-being may impair fecundity, this program also encourages research on the mechanisms controlling animal responses to physical and biological stresses that impinge upon reproductive processes. Research should contribute to an understanding of the causes, consequences, and avoidance of stress, rather than merely describing the physiological effects of stress on reproductive efficiency.

2000-01258 Ovarian Activity and Bull Management in Farmed Muskoxen
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University of Alaska Fairbanks; Institute of Arctic Biology; Fairbanks, AK 99775-7000
Seed Grant; Grant 00-35208-9168; $74,997; 2 Years

Farming of muskoxen (for wool) and reindeer (for meat and antlers) are emergent industries in Alaska. For such endeavors to be successful, it's necessary to develop fundamental husbandry tools specifically for these species. Using muskoxen in our initial studies, we will investigate the role of the bull in the onset of seasonal ovarian activity and in synchronizing estrus (heat) among the cows. The efficacy of commercially available hormonal treatments will be evaluated during the normal breeding season. Plasma progesterone concentrations will be used to interpret ovarian activity and conception will be the criterion for fertility. The seed grant will also be used to validate the use of a remote estrus detection system and to gain specialized training in follicular ultrasound. In both years, the progesterone profiles will be used to confirm the accuracy of these technologies, ultimately providing us with non-invasive tools for future use in managing the breeding season. The ability to properly manage bulls, detect and synchronize estrus provides optimal control over the breeding season, enhancing selective breeding programs, truncation of the rutting season and the ability to accurately predict and prepare for calving in the spring. The study will provide tools that can be used immediately within the industry as well as laying the foundation for research into more advanced techniques such as artificial insemination.

2000-02390 The Role of Oxidative Stress in Ovarian Follicular Development and Atresia
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Grant 00-35203-9167; $120,000; 2 Years

The follicle is the basic unit of structure and function in the ovary. Greater than 99% of all follicles degenerate and undergo atresia; however, factors that trigger atresia are unknown. It is possible that follicular atresia is caused by oxidative stress. Cells are protected against oxidative stress by oxidative stress response (OSR) proteins. Since pituitary hormones are essential for follicular growth, it is possible that expression of OSR genes is regulated by these hormones (particularly LH). The experiments in this project are designed to test 2 hypotheses. First, we hypothesize that follicular atresia is initiated by decreased expression of OSR genes. Second, we hypothesize that expression of OSR genes is regulated by LH. To test these hypotheses, ultrasonography will be used to monitor the development of individual follicles in cows. Follicles will be collected at precise, known stages of development. Expression of genes encoding OSR proteins will be measured using molecular biology techniques. We predict that

atretic follicles will exhibit low levels of expression of OSR genes, evidence of inadequate protection against oxidative stress. In contrast, we expect healthy follicles to exhibit high levels of expression of OSR genes, indicating that these follicles are protected against oxidative stress. It is also predicted that LH will stimulate expression of OSR genes, both *in vivo* and *in vitro*. These studies will help elucidate the mechanisms by which follicles avoid atresia. This fundamental information will be valuable in the development of new methods to regulate follicular growth and control fertility in domestic livestock.

2000-02410 Altering Bull Sperm Membranes to Enhance Cryosurvival

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Grant 00-35203-9136; $240,000; 3 Years

Fertility of cryopresered sperm from most dairy bulls is satisfactory, however, this is not the case for some dairy bulls possessing the highest genetic merit or for many beef bulls. Therefore, high numbers of sperm are required in each insemination dose for these bulls. This reduces the number of insemination doses produced from these bulls, far below the demand for this semen in both domestic and foreign markets. Improving sperm cryosurvival would permit fewer sperm to be needed in each insemination dose, thereby enabling higher numbers of insemination doses to be produced from these high demand bulls, in a given period of time. In addition, it would improve the fertility of all bulls in general. Because membrane damage is a pivotal problem in cryopreservation, we will study biochemically how plasma membrane lipid alterations affect sperm cryopreservation. Previous research revealed that increasing membrane cholesterol levels, via cyclodextrins, increased the number of bull sperm surviving cryopreservation. Data also indicated that liposomes composed of synthetic unsaturated lipids increase rooster sperm membrane permeability to glycerol, a potential benefit to cells during cropreservation. The objectives of this research are to: 1)determine if treating bull sperm with cholesterol-loaded cyclodextrins or cationic liposomes alters the permeability of sperm membranes to glycerol and water, and to define the membrane permeability coefficients for treated sperm; 2)using those membrane permeability coefficients, optimize cryopreservation protocols for cyclodextrin- and liposome-treated bull sperm; 3)determine if treatment with clolesterol-loaded cyclodextrins or liposomes alters the membrane fluidity of bull sperm at low temperatures; and 4)determine if treatment with cholesterol-loaded cyclodextrins or liposomes alters the ability of bull sperm to capacitate, undergo an acrosome reaction and fertilize oocytes *in vitro*. Completion of this research will improve our understanding of low temperature membrane biochemistry and function, and will facilitate improvements in cell cryopreservation in general, and bull sperm cryopreservation specifically.

2000-02406 Molecular Control of Luteal Secretion of Progesterone in Ruminants

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Grant 98-35203-6376; $200,000; 2 Years

The long-term goal of the proposed research is to enhance food production by reducing the nearly 30% early embryonic wastage which occurs as a result of inadequate secretion of the reproductive hormone, progesterone, from the corpus luteum in domestic ruminants. This problem reduces meat production by more than 400 million pounds per year in this country alone with economic losses of more than $300 million. The rate-limiting step in the secretion of all steroid hormones, including progesterone, is the transport of the steroid precursor molecule cholesterol from the outer to the inner mitochondrial membrane in steroid-producing cells. This is also the process which is most acutely controlled by regulatory processes. There are at least three proteins, steroidogenic acute regulatory (StAR) protein, peripheral-type benzodiazepine receptor (PBR), and PBR's natural ligand endosepine. The proposed research will use modern recombinant protein technologies combined with fluorescent microscopic and protein analytical procedures to determine if these proteins interact in steroid-producing cells. The role of these interactions in regulating steroid synthesis will also be evaluated. Once the basic mechanisms

involved in progesterone synthesis has been determined procedures to control this process to enhance food production will likely be possible.

2000-02156 Ontogeny and Localization of VEGF and its Receptors in the Gravid Porcine Uterus
Ford, S.P.
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Grant 00-35203-9138; $135,000; 3 Years

The long range goal of this research is to optimize litter size in economically important pig breeds. The number of live piglets farrowed is the most important component of sow productivity, and it is believed that uterine capacity is the greatest constraint on litter size. This proposal focuses on the role of the vascular endothelial growth factor (VEGF) system in modulating placental/endometrial vascularity and efficiency, which we have shown directly impacts conceptus growth, survival and litter size. The overall objective of this proposal is to characterize changes in the location and/or level of expression of the VEGF ligand-receptor system in placental and uterine tissues throughout gestation in the pig. Further, we will describe associations between VEGF and its membrane receptors with the physiological parameters of conceptus growth and function. Our guiding hypothesis is that the variation in response of individual conceptuses to similar levels of nutrient and oxygen delivery is achieved through differential modulation of placental size and/or vascularity. Further, we hypothesize that the variation in how individual conceptuses modulate the density of blood vessels at their placental-endometrial interface, and thus maternal-fetal exchange, is a result of differential expression of components of the VEGF system. Placental vascularity and growth are crucial elements in fetal development and survival in all livestock species including the pig. This proposal will provide new and vital information on the ontogeny and pattern of expression of elements of VEGF and its receptors by placental and endometrial tissues, as well as provide insight into their modulation by placental estrogens. As the VEGF system is known to have potent effects on cellular differentiation, vascular development and permeability, these insights will provide vital knowledge into factors modulating conceptus growth, survival and litter size in the pig.

2000-02398 Regulation and Function of the Uterine Mx Gene
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Grant 00-35203-9185; $80,000; 2 Years

Embryonic mortality is a major cost to production animal agriculture, costing nearly a billion dollars annually. Most embryonic wastage occurs early in pregnancy, when the embryo first signals its presence to the maternal uterus (maternal recognition of pregnancy). During this period, the free-floating embryos are dependent on uterine secretions for survival. Uterine secretions consist of, among other things, proteins and hormones which are essential for growth and development. The long-term goal of this research is to understand how the embryo, through its secretions, regulates uterine function to establish a uterine environment conducive to growth and development. This project will investigate the molecular mechanisms mediating communication between the early embryo and the uterus by examining the regulation and function of a protein termed Mx. Based on what is known about Mx and similar proteins in other tissues, it is likely that Mx is involved in the process of secretion by the uterine lining and may also be involved in protecting the lining of the uterus from viral infection (this is particularly important during insemination). Early embryos of domestic cattle, sheep and goats secrete a Type I interferon termed interferon-tau (IFN-tau). Mx is expressed in the sheep uterus throughout the estrous cycle and is strongly induced by IFN-tau in the uterine lining. This project will examine the function of Mx using cell lines derived from the sheep uterus. Specifically, studies will determine if Mx mediates secretion by the uterine mucosa. Results should determine if Mx is involved in the process of maternal recognition pregnancy by affecting uterine secretion of proteins and other hormones. These studies will provide information that will be used to improve fertility and efficiency of animal production.

2000-02279 Role (and Manipulation) of Adenosine Triphosphate in Salmonid Sperm Quality
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Strengthening Award; Grant 2001-35203-10064; $120,000; 2 Years

Adult returns of wild salmonids in North America have declined markedly over the last few decades. Accurate prediction and improvement of fish gamete quality are very important in maximizing the success of artificial fish reproduction, particularly with gametes from rare and endangered salmonids. Within this context, methodologies will be assessed to evaluate and enhance ATP levels in sperm from hatchery-reared steelhead trout and Chinook salmon, because stored ATP (and possibly phosphocreatine) is probably the primary energy source used by salmonid sperm to generate propulsion. Fresh salmonid semen samples occasionally have low motility and fertility. Because low ATP levels may be the basis for this observation, enhancement of ATP levels may increase sperm motility and fertility and hence, quality. The University of Idaho maintains a germ-plasm repository for threatened and endangered fish species. The utility of such a repository is dependent on the quality of the sperm prior to cryopreservation and the quality of the sperm after thawing. Using metabolic, motility and fertility assessments, the proposed studies will provide basic information that should result in more optimal conditions for long-term sperm storage. Finally, modest levels of CO_2 (~3%) are associated with a reversible inhibition of sperm motility probably mediated via pH. Therefore, the effects of CO_2 and pH on sperm ATP metabolism and functional characteristics will be investigated. Conceivably, short-term storage of sperm under CO_2, with subsequent CO_2 removal, may facilitate unfrozen storage of sperm.

2000-02282 Physiology and Growth Committee Conferences at the 2000 ADSA-ASAS Joint Meeting
Chase, C.C.; Houseknecht, K.L.
American Society of Animal Science and American Dairy Science Association; Savoy, IL 61874
Conference Grant; Grant 96-35203-5313; $10,275; 6 Months

The American Society of Animal Science (ASAS) and the American Dairy Science Association (ADSA) are hosting a joint national meeting from July 24-28, 2000 at the Baltimore Convention Center in Baltimore, MD. The overall goals of the conferences are for the advancement of research and knowledge by bringing together scientists to foster scientific exchange, update information, and to identify future research needs. The Physiology and Endocrinology Committee and the Growth and Development Committee have organized the conferences proposed jointly and by each committee. The topics of the mini-symposia include: 1)Luteal cell interactions and function, 2)Molecular mechanisms of hormone action, and 3)Appetite regulation: leptin and beyond. There will also be a session devoted to the impact of clinical/subclinical disease on animal performance. In each of the mini-symposia, three or four invited speakers will give presentations (30-40 minutes) that will be followed by short presentations (15 minutes each) from selected, submitted abstracts. Anchoring these mini-symposia, and of most interest to animal scientists, will be a full day symposium on genetics titled, “From Genome to Function: Application of Genomics/Functional Genomics to Animal Agriculture”. Invited speakers will present state-of-the-art research on techniques, applications to animal agriculture, and legal/regulatory issues. This multi- Animal Health not finalized

2000-02143 Role of Oocyte Metabolic Activity in the Control of Porcine Embryo Development
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Grant 00-35203-9379; $200,000; 3 Years

The female gamete, or oocyte, is an integral part of the reproductive process as it not only contains the female genetic contribution but also the entire component of cellular machinery necessary to support early embryonic development. Maturation of the oocyte, which describes progression from the immature state in which it is arrested inside the ovarian follicle to the completion of the first reduction division of

meiosis when the oocyte can be fertilized, is an extremely important and sensitive period. Cellular events occurring during this time profoundly influence the viability of the resulting embryo. The biochemical processes of cellular energy metabolism are likely to play a significant role in the acquisition of developmental competence in oocytes. Our goal is to investigate the role of metabolism in the regulation of developmental competence of porcine oocytes, and to define parameters of energy substrates and metabolism that are correlated with increased developmental potential. Our specific objectives are to examine the metabolic activity and energy production of porcine oocytes matured both in the laboratory and inside the pig, and to investigate the relationship of oxygen radical reducing agents and metabolism with oocyte maturation and developmental potential. These experiments will increase our understanding of oocyte metabolic processes and developmental regulation, as well as provide information to improve *in vitro* embryo production. Understanding how metabolism regulates developmental competence in porcine oocytes will allow us to maximize developmental potential of oocytes for use in pharmaceutical protein production and xenotransplantation programs in addition to agricultural and research applications.

2000-02151 Formation and Treatment of Ovarian Follicular Cysts in Dairy Cows
Silvia, W.J.
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Strengthening Award; Grant 00-35203-9174; $160,000; 4 Years

The overall objective of the experiments to be conducted in this project are to study the causes of ovarian follicular cysts in dairy cows and to develop new preventative measures and treatments. In normal dairy cows, follicles develop on the ovary and either ovulate to release an egg for fertilization or degenerate. In cystic cows, the follicles fail to ovulate but continue to grow to an abnormally large size. These cystic ovarian follicles secrete steroid hormones that disrupt normal reproductive cycles. Approximately 15% of all dairy cows form ovarian follicular cysts each lactation period. Current treatments are only effective in 53% of cases. In experiment 1, we will determine if abnormal secretion of progesterone is associated with the formation of cysts. In experiment 2, we will determine how the lowest concentration of progesterone that is required to disrupt normal reproductive cycles and induce formation of ovarian follicular cysts. In experiment 3, we will test one potential treatment for ovarian follicular cysts in dairy cows.

2000-01156 Ultrasound Scanning System
Silvia, W.J.
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Equipment Grant; Grant 00-35208-9176; $10,600; 1 Year

The major research focus of our laboratory has been to study the causes of ovarian follicular cysts in dairy cows and to develop new preventative measures and treatments. In normal dairy cows, follicles develop on the ovary and either ovulate to release an egg for fertilization or degenerate. In cows with cystic ovarian follicles, the follicles fail to ovulate but continue to grow to an abnormally large size. The cystic follicles secrete steroid hormones that disrupt normal reproductive cycles. Approximately 15% of all dairy cows form ovarian follicular cysts each lactation period. Current treatments are only effective in 53% of cases. One essential piece of equipment needed to study ovarian follicular cysts is an ultrasound scanning system. This device enables the researcher to non-invasively measure follicle size repeatedly on the same animal for months at a time. Using this system, we can document the formation of ovarian follicular cysts and the effectiveness of experimental treatments. Funding from this proposal will be used to purchase an ultrasound scanning system that will be devoted primarily to the study of ovarian follicular cysts in dairy cows.

2000-02146 Gonadotropin and Their Receptors in Channel Catfish
Trant, J.M.

University of Maryland Biotechnology Institute; Center of Marine Biotechnology; Baltimore, MD 21202
Grant 00-35203-9105; $200,000; 2 Years

In many cultured species of fish, the maturing eggs (ovarian oocytes) will not fully develop in captive broodstock thereby resulting in reproductive failure. In general, this problem can be tempered with injections of the appropriate hormones to induce spawning, although success is highly variable. The working hypothesis of this proposal is that the seasonal and differential regulation of the genes encoding the gonadotropin (FSH and LH) subunits in the pituitary and the genes encoding gonadotropin receptors (FSH-R and LH-R) in the ovary control gonadal physiology including competence of the oocytes to mature and ovulate. As a first step in testing this hypothesis, the cDNA gene products encoding the two receptors have been isolated by this lab from the channel catfish (*Ictalurus punctatus*) and shown to be correlated with spawning in catfish. This proposal will extend these findings with this model species using molecular techniques in order to understand the seasonally-regulated changes associated with oocyte growth and maturation. Specifically, the catfish receptors will be characterized and the hormone-binding characteristics will be evaluated in a cell culture system. In a concurrent effort, the cDNAs encoding the two gonadotropin beta-subunits will be isolated. These cDNAs will serve as probes to determine the seasonally-regulated production of the gonadotropins and their receptors correlated with a multitude of morphological and endocrine parameters. Due to the conserved nature of the GtH-Rs and reproductive endocrinology, these results should be applicable to many other fish species. This study will provide an important foundation for studies of gonadal physiology and the development of assays for evaluating the reproductive status of brood stock in an aquaculture situation and thus improve efficiency in fish production.

2000-02391 Inhibin Regulation of Intrafollicular Estradiol Production
Ireland, J.J.; Pursley, J.R.
Michigan State University; Department of Animal Science; East Lansing, MI 48824-1225
Grant 00-35203-9117; $220,000; 3 Years

Inhibin exists as a family of proteins of diverse sizes produced primarily by granulosal cells in ovarian follicles. We hypothesize that alterations in intrafollicular concentrations of inhibin antagonize growth and differentiation of dominant and subordinate follicles during each non-ovulatory follicle wave in cattle. To test this hypothesis, the following two aims are proposed: 1) to determine if the suppressive action of inhibin on estradiol production by granulosal cells is altered during development of dominant and subordinate follicles, and 2) to examine the specificity of the suppressive action of inhibin on granulosal cells. To accomplish these goals, a combination of *in vivo* and *in vitro* approaches will evaluate the effects of inhibin on basal and gonadotropin-induced estradiol, progesterone and (or) activin production during different stages of development of the first-wave dominant and subordinate follicles in heifers. Collectively, results of our studies will provide new insight into the physiological role of inhibin on growth and differentiation of dominant and subordinate follicles in the first follicle wave in heifers. In addition, this new information will characterize alterations in the suppressive action of inhibin during a follicle wave, which could improve the usefulness of inhibin-based therapeutic agents to regulate follicle growth in cattle. Since estradiol is a key hormone involved in numerous reproductive processes including follicular growth, estrus, onset of preovulatory gonadotropin surges, and uterine function, understanding how estradiol production is regulated in dominant follicles may provide new information important for design of better methods to control the estrous cycle and improve fertility.

2000-02141 Neuroplasticity and GnRH Secretion
Billings, H.J.
University of Michigan; Reproductive Sciences Program; Ann Arbor, MI 48109-0404
Postdoctoral Fellowship; Grant 00-35203-9183; $90,000; 2 Years

This study addresses a novel mechanism for regulating estrous cyclicity in a domestic animal species, structural remodeling of the gonadotropin-releasing hormone (GnRH) neurosecretory system. In

particular, the proposal focuses on the interaction of the polysialylated (PSA) form of neural cell adhesion molecule (NCAM) with the GnRH secretory system near the time of ovulation in the sheep. A few regions of the adult brain retain expression of PSA-NCAM, a molecule known to promote neural changes during embryonic development. These regions include areas rich in GnRH neurons where changes in the association of GnRH neuronal terminals with the pituitary portal vasculature have been observed during the estrous cycle. Thus, PSA-NCAM may remain functional in the adult as part of the mechanism by which neural remodeling occurs in the GnRH system. Therefore, the purpose of this proposal is to test the following overarching hypothesis: Estradiol-induced changes in GnRH secretory patterns (pulsatile and surge) during the follicular phase of the cycle are mediated by changes in the association of PSA-NCAM with GnRH neuronal fibers and terminals in the median eminence. This proposal includes two specific aims to test this hypothesis: 1) Does the expression of PSA-NCAM in the median eminence, or its association with the fibers and terminals of GnRH neurons, change during the follicular phase consistent with the pattern of changes in GnRH secretion into pituitary portal blood? 2) Is PSA-NCAM needed for the patterns (pulsatile and surge) of GnRH and LH secretion during the follicular phase?

2000-02127 Modulation of Reproductive Efficiency by Prolactin in the Domestic Turkey

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Grant 00-35203-9157; $190,000; 3 Years

The reproductive efficiency of turkeys is low in comparison to chickens. One component of this low efficiency is relatively poor egg production, which is related to the incidence of the expression of incubation behavior (broodiness) by turkey hens. Convincing evidence have been presented implicating increased prolactin secretion as a cause of ovarian regression that results when turkey hens shift from egg laying to broodiness. We proposed a research strategy aimed to clarify the mechanisms involved in the increased prolactin secretion, and the mechanisms by which elevated prolactin levels induce ovarian regression and termination of egg laying in the turkey. Studies involving the use of electropharmacological and immunocytochemical techniques will be used to reveal the prolactin-releasing pathway within the brain; and to localize the neurons that produce the two-brain chemicals (dopamine, vasoactive intestinal peptide) known to regulate prolactin secretion. Further, we plan to investigate the intracellular pathway(s) used by dopamine and vasoactive intestinal peptide to regulate prolactin gene transcription utilizing tissue culture and molecular biology techniques. Insight into brain and pituitary mechanisms regulating high prolactin and the termination of egg laying is critical for the advancement of knowledge about avian reproduction and for pursuit of improved reproductive efficiency in the domestic turkey.

2000-02397 Interval to Ovulation in Weaned Sows

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Grant 00-35203-9106; $180,000; 2 Years

Sows must return to estrus after weaning so that they can be inseminated and establish a new pregnancy. Even under optimal conditions, the variation in the interval to estrus after weaning is far too great. Furthermore, sows must be inseminated twice to insure optimal conception rate and litter size because ovulation is not precisely timed relative to the onset of estrus. The poor timing of reproductive events places significant demands on labor for estrus detection and insemination and wastes semen because sows are inseminated two or more times. We believe that synchronized periods of follicular development (follicular waves) occur in sows before weaning. Weaning is a random event relative to the follicular wave. Therefore, that the variation in weaning to estrus interval in sows is caused by weaning at random times of the follicular wave. The objective of this application is to characterize the mechanisms that lead to follicular waves before weaning in sows. If our model is correct then the traditional model for follicular growth before weaning in sows needs to be changed to a new model that includes synchronized

follicular development. The new model changes the way we think about managing follicular populations in sows and opens up the possibility of reducing the variation in the interval from weaning to estrus or ovulation by synchronizing follicular growth before weaning. This would benefit the producer by shortening the period of estrous detection and insemination and possibly eliminate the need for double inseminations if a precise synchronization of ovulation can be achieved.

2000-02163 Developing Precise Methods of Estrous Cycle Control for Beef Cows and Heifers

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Grant 00-35203-9175; $200,000; 3 Years

Precise control of estrous cycles in cattle requires the manipulation of both follicular waves and luteal lifespan. Two widely used methods of synchronizing estrus in beef cows and heifers include the GnRH-PG (GnRH followed 7 days later by $PGF_{2\alpha}$ [PG]) and MGA-PG (0.5 mg MGA/hd/day for 14 days and PG on day 17 after MGA withdrawal) protocols. Each of these protocols have drawbacks that preclude them from providing the degree of synchrony required for successful fixed-time AI. We hypothesize that sequential treatment of MGA, GnRH, PG synchronizes estrus in a way that facilitates fixed-time AI. Specific Aim 1 is designed to determine a long-term treatment that provides the highest estrus response and best synchrony of estrus among postpartum cows. Specific Aim 2 is designed to compare two short-term treatments, 7-11 Synch (7 days of MGA, PG on day 7, GnRH on day 11, and PG on day 18) and GnRH-GnRH-PG (GnRH followed in 7 days by GnRH and PG 7 days later) to identify the treatment that provides the highest estrus response and best synchrony of estrus in cows. For heifers, the best long-term treatment will be compared with 7-11 Synch. Specific Aim 3 will compare the best long-term treatment and the best short-term treatment with CO-Synch in cows. Fixed-time AI will be performed in heifers comparing the best long-term treatment with 7-11 Synch. Treatments will be compared on the basis of results from fixed-time AI for both cows and heifers assigned to each treatment.

2000-02160 Ovarian Follicular Development in Cattle: Activation and Growth *in vitro*

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Grant 00-35203-9151; $200,000; 2 Years

In mammalian ovaries, most oocytes reside in non-growing, primordial follicles. Virtually nothing is known about the mechanisms that regulate movement of these follicles into the growing pool (activation). The pool of resting primordial follicles is a resource, as yet untapped, that could be exploited as a source of material for *in vitro* fertilization to hasten genetic improvement of domestic species, preserve endangered species, and provide alternative methods for alleviating infertility in women. This proposal has two complementary goals: 1) to investigate the signals that regulate the activation primordial follicles and 2) to develop methods for inducing further growth of primordial follicles activated *in vitro*. When pieces of bovine ovarian cortex are cultured *in vitro*, almost all the primordial follicles begin to grow, but when bovine cortical pieces are grafted beneath the chorioallantoic membrane of chick embryos, the wholesale, spontaneous activation observed *in vitro* is completely absent. We propose to use these two complementary experimental systems to address fundamental questions about the signals that control activation of primordial follicles and the primary to secondary follicle transition. We will test specific hypotheses about the potential of our two culture systems to support development of primordial and primary follicles (Specific Aim #1), the effects of a potential stimulator (kit ligand) on activation of primordial follicles (Specific Aim #2), and the effects of insulin on development of activated follicles. These experiments are crucial steps towards the ultimate goal of using the large numbers of primordial follicles in mammalian ovaries to hasten genetic improvement of domestic animals.

2000-02412 Function and Regulation of the Inhibin/Activin Subunits in the Domestic Hen
Johnson, P.A.
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Grant 00-35203-9116; $180,000; 2 Years

Our overall goal is to understand the regulation of inhibin and activin and the role that these hormones play in ovarian follicle development in the domestic hen. The unique hierarchical arrangement of ovarian follicles in the hen provides a model system to study the regulation of follicle development. Knowledge about the function of ovarian inhibin and activin in a species with such predictable follicle development may permit generalization to other domestic species. Previous data have indicated the presence of inhibin subunit RNA as well as bioactive inhibin in the ovarian granulosa layer of the domestic hen. In addition, we have observed dramatic changes in regulation of the inhibin/activin subunit levels throughout follicle development. The most marked changes in expression of these subunits occur in the small, growing follicles. The importance of these changes with respect to follicle selection and development, as well as the possible role of the oocyte factor, growth/differentiation factor 9 (GDF-9), have not previously been examined. To address these areas, three Specific Aims are proposed. In Specific Aim I, we will evaluate the role of Inhibin B in follicle selection and development in the small follicles of the ovary. The purpose of Specific Aim II is to characterize the expression of GDF-9 with respect to follicle development. Finally, in Specific Aim III, we will determine if there is a correlation between follicle health and expression of the beta B-ubunit and GDF-9. These ovarian hormones may be important in regulating follicular recruitment and development.

2000-02165 Early Maturation of the Endocrine Axis in Heifers
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Grant 00-35203-9133; $200,000; 3 Years

Puberty typically occurs between 12 and 24 months of age in heifers. The understanding of hormonal changes that immediately precede puberty and the influence of environment on age of puberty are extensive. However, a substantial proportion of heifers fail to reach puberty before their first breeding season, which negatively impacts their lifetime reproductive efficiency. In contrast, some heifers have precocious puberty at 4-8 months of age; resulting in costly, unwanted pregnancies. Furthermore, nutritional status during the period from 3-7 months of age can significantly influence incidence of precocious puberty and age at puberty. The early period of sexual maturation, from birth to 8 months of age, and the nutritional status during this period appears to be an important determinant of age at puberty. However, limited information exists to define the reproductive mechanisms that control, and the impact of nutrition on, the early maturation of the reproductive axis. The goal of this project is to begin to define the process of sexual development during the early maturational period in heifers. We hypothesize that the reproductive system of all heifers is mature by 6-7 months of age in all females but functionally restrained in most heifers by negative feedback of estradiol on the secretion of LH. The objectives of the project are to describe the hormonal changes associated with precocious puberty and the impact of nutrition on these changes; and to determine the fluctuations in the negative feedback of estradiol on LH secretion during the early maturational period.

2000-02132 Neuroanatomical Basis of Pulsatile GnRH Release in the Ewe
Lehman, M.N.; Goodman, R.L.
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Grant 00-35203-9159; $200,000; 2 Years

Key to understanding how farm animals reproduce, is to understand how the brain controls the secretion of hormones that regulate fertility. One such essential hormone is gonadotropin-releasing hormone (GnRH). GnRH is the master hormone controlling reproduction. GnRH is made by brain cells

and carried by specialized blood vessels from the brain to the pituitary gland, where it, in turn, controls the secretion of hormones controlling gonadal function. GnRH plays a critical role in regulation of fertility in farm animals including sheep. Changes in the activity of GnRH brain cells are responsible for the estrous cycle, and the shut down of reproduction that occurs during seasonal anestrus and prior to puberty. Our long-term goal is to identify neurotransmitter systems in the brain that control the activity of GnRH cells. One such transmitter system contains endogenous opioids; opioids act in the brain to alleviate pain and stress, but are also important regulators of GnRH activity. In the previous period of USDA support, we identified a specific type of opioid system, the dynorphin-kappa system, and showed that it plays a key role in regulating GnRH cell activity during the estrous cycle. In the proposed experiments, we will determine if these opioid neurons are directly influenced by progesterone, a hormone that is elevated during the luteal phase of the estrous cycle. This research will help reveal the means by which peripheral hormones normally stimulate GnRH secretion. Because changes in GnRH secretion are critical in controlling sheep reproduction, this knowledge will allow us to develop new ways of maximizing reproductive efficiency in sheep and alleviating inefficiencies in lamb production.

2000-00483 Reproductive Tract Biology, Gordon Research Conference
Fisher, S.J.
University of Rhode Island; Gordon Research Conferences; West Kingston, RI 02892-0984
Conference Grant; Grant 00-35203-8849; $10,000; 1 Year

The requested funds will be used to partially defray the travel and registration costs of participants in the Reproductive Tract Biology Gordon Conference to be held July 2-7, 2000. As compared to the numerous other scientific meetings held every year, several aspects of the Gordon conferences are unique. First, the number of participants is limited to 120. Since there are many more applicants than attendee slots, the conference organizers can select the leaders in the field. Second, there is also a longstanding tradition of encouraging the attendance of junior colleagues; the participation of graduate students and postdoctoral fellows is strongly encouraged. There is also a large emphasis on including women and minority investigators. Third, this unique mix of senior and junior scientists offers an unparalleled opportunity for the exchange of information. The meeting has a particularly large impact on the careers of entry level investigators. There is a long tradition of participation of USDA grantees and their students in this meeting, both as attendees and as conference chairs. The year 2000 conference continues this tradition by highlighting research that is done in large animal models with the goal of improving the fertility of domestic animals, an important topic at the beginning of the new century. This Gordon Conference attracts participants from a wide variety of fields and backgrounds ranging from basic science to clinical practice, including veterinary medicine. Accordingly, the principal goal of this conference is to stimulate cross-disciplinary exchange and integration of information concerning the reproductive tract.

2000-00482 Mammalian Gametogenesis and Embryogenesis, Gordon Research Conference
Kidder, G.M.; Hunt, P.A.
University of Rhode Island; Gordon Research Conferences; West Kingston, RI 02892-0984
Conference Grant; Grant 00-35203-8961; $3,000; 1 Year

The field of mammalian gametogenesis and embryogenesis (the production of gametes and embryos) has seen a resurgence in the past two decades because of developments in reproductive technology. Strategies such as gamete or embryo transfer, germ line transplantation, gene injection, and cloning promise to accelerate the improvement of livestock genetics. The 2000 Gordon Research Conference will examine the scientific underpinnings of reproductive technologies, explored through research on both laboratory and domestic animal models. Sessions will focus on stem cells, the signals that control gamete and embryo development, the metabolic requirements of gametes and embryos, the genomes of gametes and embryos, and techniques for manipulating the genetic makeup of embryos. It is expected that unpublished, state-of-the-art information will come forth at the conference that will help to prime the next step in genetic improvement of livestock.

2000-01150 Purchase of a High Resolution Fluorescence Light Microscope

Evenson, D.P.
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Equipment Grant; Grant 00-35208-9158; $24,811; 1 Year

This new, high resolution, state-of-the art light microscope will be used to determine the pathology, primarily in the genetic material, of mammalian sperm that are subfertile. Our lab has developed a test that shows what percent of sperm in a semen sample carries an abnormality. With the use of fluorescent probes, that can be observed in this new high-powered microscope, we will be able to help unravel the nature of the pathology that leads to subfertility. Of primary interest is to determine the extent of fragmented DNA in the nuclei as determined by using specific fluorescent stains that can be seen well only with the improved optical components of this light microscope. We will also attach a computer controlled image capture system so that various morphological features can be automatically scored for a population of sperm in an ejaculate. These light microscope assays will provide data as to whether to cull selected semen samples or to cull the bull or boar from the breeding herd. Secondly, this microscope will be used to study the pathology of the porcine reproductive and respiratory syndrome virus (PRRSV) that is the most economically important disease of swine in the United /States and the rest of the world's swine population. Studies are planned with this microscope to identify the tissues and cell types supporting this virus replication. The digital camera attached to this microscope will allow a rapid comparison between various morphometric tests by storing digital images that can be placed in grids on the computer for comparison.

2000-02395 Molecular Probes of Bull Sperm Nuclei Producing Abnormal Embryos

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Strengthening Award; Grant 2001-35203-10063; $100,000; 2 Years

The overall objective of this project is to further define the cellular and molecular characteristics of sperm chromatin structural abnormalities that lead to poor reproductive outcomes in cattle. The percentage of sperm with abnormal DNA, detected by the Sperm Chromatin Structure Assay (SCSA), has been highly correlated with fertility in a variety of species, including cattle. In order to understand the etiology of such sperm chromatin abnormalities and to identify their impact on early embryo development, the molecular and cellular features of sperm containing various levels of chromatin damage will be investigated. Normal and SCSA-defined abnormal sperm will be sorted from individual samples by flow cytometry and further characterized for DNA strand breaks, protamine characteristics, and image analysis. Other flow cytometric and biochemical protocols will analyze sperm samples for DNA-protein configurations in semen populations known to have high percentages of SCSA-defined sperm chromatin defects. The effect of bull sperm, which possess a high incidence of chromatin damage, on subsequent early embryonic differentiation and development will be determined *in vitro*. Preliminary data suggest that sperm with abnormal chromatin are capable of fertilizing oocytes but do not provide adequate support for normal embryonic development. Semen samples with a very low and a high level of chromatin abnormalities will be used for *in vitro* fertilization of bovine oocytes. The resulting embryos will be scored to evaluate whether the abnormal chromatin measured in the two groups of good and poor semen has a negative effect on embryonic development.

2000-02290 Roles of Fibroblast Growth Factor-7 in Uterine Biology and Pregnancy in Pigs

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Grant 00-35203-9137; $190,000; 2 Years

Fibroblast Growth Factor-7 (FGF7) is normally produced by stromal cells to regulate proliferation and function of epithelial cells of various organs, including the uterus. In pigs, however, FGF7 is expressed by uterine lumenal and/or glandular epithelial cells and binds to its receptor (FGF Receptor 2IIIb) on those same cells. FGF Receptor 2IIIb is expressed by uterine epithelial cells and conceptus membranes (trophectoderm/chorion) of pig conceptuses (embryo and extraembryonic membranes). Thus, FGF7 mediates interactions between uterine epithelial cells and between uterine epithelial cells and trophectoderm/chorion of conceptuses to induce proliferation and differentiated functions for establishment and maintenance of pregnancy. The proposed studies will test the hypothesis that FGF7 is expressed by epithelia of pig uterus in response to estrogens and/or interleukin-1 from conceptuses to stimulate:1) proliferation and differentiated functions of uterine epithelial cells; (2) proliferation and differentiated functions of trophectoderm/chorion (paracrine effects); and (3) expression of trophectoderm/chorion genes responsible for signaling pregnancy recognition and establishment of pregnancy. This research will determine:1)effects of progesterone, estrogen, and IL-1b on expression of FGF7 by uterine epithelium during the period that conceptuses attach to the uterine lumen for implantation; 2) effects of FGF7 on proliferation and differentiated functions of uterine epithelia necessary for conceptus development and implantation; 3) effects of FGF7 on trophectoderm/chorion cell proliferation and differentiated functions essential for conceptus development and establishment and maintenance of pregnancy. Knowledge of the roles of FGF7 in the pregnant uterus will be used to develop management strategies to enhance reproductive efficiency in swine.

2000-02274 Leptin As a Metabolic Gate to the Central Reproductive Axis in Heifers

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Texas Agricultural Experiment Station; College Station, TX 77843-2147
Grant 00-35203-9132; $200,000; 3 Years

Nutrition and body energy reserves (fatness) exert profound effects on reproduction in mammals, including cattle. These effects include modifications in age at puberty and reproductive efficiency after calving. However, how nutrition regulates these functions is not well-understood. Within the last 6 years, leptin, a hormone produced and secreted primarily by fat cells, has become a plausible candidate for the chemical signal which communicates nutritional status to the brain, thus regulating the timing of onset of fertility. Until recently, neither the materials necessary to accurately measure leptin activity, nor a source of purified leptin for use in animal experiments, has been available. However, during the past 3 years, one of the principal investigators (PI #2) of this project has been able to develop materials to accomplish these objectives. Using these materials, PI #1 has conducted experiments in cattle showing that the leptin gene and circulating leptin are responsive to changes in nutrition, that circulating leptin increases during sexual maturation, and that injection of purified leptin into the brain stimulates reproductive (luteinizing hormone), growth (growth hormone), and metabolic (pancreatic insulin) hormone secretion. Based on these preliminary findings, the long-term objectives of these experiments are to gain a better understanding of the role of leptin as a nutritional signal to the brain during and after sexual maturation. Specific objectives are to determine the role of leptin in regulating onset of sexual maturation of heifers subjected to normal and growth-restricted diets.

2000-02150 Down Regulation of Gap Junctions in the Ovarian Follicle: Is it Required for Maturation or Ovulation?

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Grant 00-35203-9135; $200,000; 2 Years

Aquaculture contributes about 20 percent of the total fisheries production worldwide, but there is a need for this contribution to increase in order to keep up with the growing demand for fish products. However, the expansion and diversification of aquaculture are to a large degree dependent on the reliable and adequate supply of fingerlings, which is a current problem for the industry. Improved knowledge of

the basic physiology of ovarian maturation and ovulation is therefore necessary for the development of better reproductive technologies of relevance to aquaculture. The program goal of this research is to determine the hormonal control of ovarian processes that are key to proper egg development and fingerling production in fishes. Some of the specific processes to be investigated during this research include physiological, biochemical and molecular mechanisms of cell-to-cell communication in the ovary. In particular, we will conduct original research on the role of this communication during the process of ovulation. The animal model for this research is the Atlantic croaker, perhaps the most well-established fish model available for this type of research. Our conceptual model is a two-stage model of hormonal control of ovarian maturation, a model designed by us primarily on the basis of our previous work with the Atlantic croaker. The results of this study are expected to advance current knowledge of the basic mechanisms controlling egg production, and this enhanced knowledge is in turn expected to contribute to better reproductive technologies for aquaculture.

2000-02154 Annual Meeting of the Society for the Study of Reproduction

Eppig, J.J.
Society for the Study of Reproduction, Inc; Madison, WI 53711-2021
Conference Grant; Grant 96-35203-5311; $10,000; 7 Months

The annual meeting of the Society for the Study of Reproduction (SSR) will be held July 15-18, 2000 in Madison, WI. The SSR is the major professional society for scientists involved in research in the basic science of reproduction. Organized around the theme of "Reproductive Sciences: A Forecast for the New Millennium", the meeting is expected to draw over 1000 participants. Presentations will include: a Keynote Address, the President's Symposium, 3 State-of-the-Art Lectures, 15 minisymposia, and poster and oral sessions involving presentations by the attendees. The Keynote Address will focus on issues that are directly relevant to the future of research on domestic animals. The minisymposia will include a wide variety of topics including: Mechanisms of Reprogramming in Nuclear Transplantation; Angiogenesis in the Uterus and Placenta; Immune Privilege in the Pregnant Uterus; Fetal Origins of Adult Disease; Pulses, Peptides, and Promoters: Differential GnRH Actions on the Pituitary; Animals and Scientists: Partners for Progress in Reproductive Biology; Neuroendocrinology; Selection and Maintenance of the Dominant Follicle; Cell Signal Transduction: Shall it Be Life or Death? The new millennium will surely bring on an unprecedented flow of new information regarding the genetic control of reproduction in domestic animals; the challenge is to bring this wealth of knowledge to the production of animals of agricultural importance. Domestic animals are of critical importance in providing human food, in the manufacture of clothing and pharmaceuticals, etc.; thus, understanding and enhancing reproductive efficiency in animals of agricultural importance is an important goal for reproductive biologists.

2000-02159 Angiotensin II Regulation of Uterine Artery Endothelial Vasodilator Production in Pregnancy

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Grant 00-35203-9189; $120,000; 2 Years

During pregnancy blood flow to the uterus increases to match the increasing demands of the growing fetus. These changes occur, in part, through the increased production of vasodilators to relax the blood vessels. Results from our recently established cell culture model have shown that the cell signaling mechanisms underlying changes in blood flow are different from what was traditionally expected. The aim of this grant is to test whether the responses seen in the cell culture model maintained outside the animal for 14 days are similar to the results obtained from freshly isolated cells. We have found that on activation of cells with the vasoconstrictor AII there is a change in level of calcium and movement of the protein ERK1/2 to the nucleus as well as production of the soluble gas NO (a vasodilator). Using sophisticated microscopic imaging techniques we can now observe whether similar changes occur in freshly isolated single cells. We can also investigate changes in NO production if we block either the

ERK1/2 or calcium pathways of cell signaling. Responses will also be measured in pregnant and nonpregnant animals. In this way we can then understand how these signaling pathways may be perturbed in abnormal pregnancies resulting in growth retardation and endangering the lives of newborn animals. Our long-term goal is to develop strategies to prevent growth retardation and maximize reproductive efficiency.

2000-02276 Mechanisms Involved in Acquisition of Luteolytic Capacity

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Grant 00-35203-9134; $300,000; 3 Years

High reproductive efficiency is critical for profitability of livestock operations. Decreased reproductive efficiency can result from non-optimal function of the ovaries. Conversely, livestock producers can increase reproductive efficiency by manipulating the function of the ovaries so that livestock have optimal fertility at the best time for livestock profitability. One structure on the ovary that is critical for reproductive efficiency is the corpus luteum (CL). Function of the CL is crucial in normal maintenance of pregnancy. Manipulation of CL function by using commercial products that contain prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is the most common method that is currently used for synchronization of cattle. In this research project 6 experiments will be performed to evaluate the mechanisms involved in determining the responsiveness of CL to $PGF_{2\alpha}$. The first 2 experiments will determine the normal changes that occur when a cow acquires the capacity to respond to $PGF_{2\alpha}$. The second 2 experiments will evaluate whether production of $PGF_{2\alpha}$ within the CL is important for the response of CL to injected $PGF_{2\alpha}$. The third set of 2 experiments will determine if estradiol from follicles is the critical hormone that causes the CL to be responsive to $PGF_{2\alpha}$. It is anticipated that this basic research information can be used to increase pregnancies by preventing premature regression of CL in pregnant livestock. This basic information may also improve the efficiency of $PGF_{2\alpha}$ use by livestock producers.